Effect of Adenosine Triphosphate on *in vitro* Fertilization in Mice as a model for Human Being

Muhannad A. Mashkor[1], Muhammad-Baqir M-R. Fakhrildin[2],Nawal K. Al-Ani[2]

1- Department of Applied Embryology- High Institute of Infertility Diagnosis and Assisted Reproductive Technologies- Al-Nahrain University-Baghdad, IRAQ

2-Department of Clinical Reproductive Physiology- High Institute of Infertility Diagnosis and Assisted Reproductive Technologies- Al-Nahrain University-Baghdad, IRAQ

Abstract

Back ground:
Optimal IVF and embryo culture conditions depend on the composition of culture media being the most important determinants of successful in vitro interaction of the gametes and subsequent embryo development. ATP is fundamental factor to maintain the life, by providing energy, and controlling the cell function and metabolism.

Objective:
To investigate the effect ATP supplied to culture medium on fertilization rate and in mice as model for human being.

Materials and Methods:
The female mice (90) were superovulated using superovulation program (SOP) to produce large number of oocyte were divided into three groups, G1 inseminated with vassal sperm *in vitro* alone (control) , G2 inseminated with vassal sperm *in vitro* using SMART enriched with ATP(1.25mM). While, G3 inseminated with vassal sperm *in vitro* using SMART medium enriched with ATP (2.5mM). Mouse spermatozoa were collected from both vas deferens. Then sperm parameters were assessed after 30 min. IVF technique was performed for 3 groups.

Results: The results of the present study appear, significantly increment ($P<0.05$) in the IVF (%) was observed when using 2.5 mM ATP as compared to 1.25 mM ATP and control group.

Conclusions: The result showed that addition of high ATP concentration (2.5 mM) enriched to the culture medium improved percentage of *in vitro* fertilization.

Key words: ATP, *in vitro* fertilization,mice.

Introduction:

In vitro fertilization is a procedure that involves retrieving oocytes and spermatozoa from the female and male respectively, then, placing them together in a laboratory dish to facilitate fertilization. The intrinsic quality of an oocyte is the key factor determining its developmental competence [1.2.3].

Starting from fertilization and ending with implantation, pre-implantation embryo development can be divided into several well-orchestrated stages: zygote (one cell), two cell, four cells, eight cells (cell cleavage), morula and blastocyst. In mammals, pre-implantation development is characterized by various morphological and physiological transitions that occur after fertilization [2]. Once the sperm has fertilized the oocyte, the centriole and microtubules arising from the sperm bring the male and female pronuclei into juxtaposition [4].

Mitochondria are the major source of energy in eukaryotic cells, producing adenosine triphosphate (ATP) via oxidative phosphorylation and the citric acid cycle [5,6]. Adenosine 5'-triphosphate (ATP) is a purine nucleotide found in every cell of the human body.[7,8]

Adenosine 5-triphosphate is a fundamental factor to maintain the life, by providing energy and controlling the cell function and metabolism. In spermatozoa , ATP plays important roles for the movment to femal reproductive tract, vability and penetration to fertilize with oocyte [9]. The objective of this study to to investigate the effect of ATP supplied to culture media on rate of *in vitro* fertilization in mice as a model for human being.

Materials and Methods:

Forty mature male and 90 females mice with age of 8-1o weeks old and 25-30 gm body weight were obtained from the Animal House at the High Institute of Infertility Diagnosis and Assisted Reproductive technologies /AL-Nahrain University. They were kept in an air conditioned room (~25°C) with range a 12-14 hours photoperiod. The animals were housed in a standard cage of opaque plastic measuring (29×15×12) cm. Cages floor covered with wooden shave. Each cage contains four animals and tap water and diet were freely available for the animals. The isolated females were kept in separate cage to make sure there is no meeting between them happened and no pregnancy taking place by sexual intercourse. The examined clearly in every week, abnormal and sick mice were excluded from the experiment. The animal, cages were cleaned and sterilized with 70% ethyl alcohol once a week regularly.

Preparation of adenosine triphosphate (ATP):

Preparation of ATP powder (ATP molecular weight 551.15; Rashmi Diagnostics, India) was used for preparation of ATP stock solution by dissolving 0.1377g in 5 mL of SMART medium with final concentration this stock 50mM. For preparation of low concentration treated group (G2; 1.25mM), 0.25mL of stock solution was diluted with 9.75ml of SMART medium. While 0.5 ml from stock solution add to 9.5 mL of SMART for preparation of high concentration treated group (G3; 2.5mM) .However, alone was consider SMART medium for control group (G1) only.

Superovulation induction:

Super Ovulation is a routine procedure for producing greater yields of oocytes, which can be done after the administration of exogenous gonadotrophins to female mammals. Superovulation program starts by injecting female mice with 7.5 I.U. of PMSG (intraperitoneally), Also second injection with 7.5 I.U. of PMSG after (24) hours, after (47-48) hours from 2nd injection, 3rd injection 15 I.U. of hCG. Sacrificing

female and oocytes were recovered (16-18) hour post-hCG by flushing the oviducts [10].

***In Vitro* Sperm collection:**

Sacrificing male mice approximately at morning day of ova collection. Spermatozoa collected from both vasdeferens by flushing of vas deferens using one mL of SMART medium. Push the SMART medium (0.5 ml) through vas using insulin syringe, the sperms were taken out, and then discard the vas deference. The collected sperm were incubated for at least 30-60 minutes to achieve capacitation.show table (1)

Sperm parameters:

Sperm concentration:

A drop of 10µl spermatozoa suspension with SMART medium was placed on a microscopic slide and covered with a cover slip (22x22) mm. Concentration of spermatozoa (sperms/million) was calculated from the mean number of spermatozoa in ten high power microscopic fields under magnification of (400x).

Sperm concentration=No. of spermatozoa x the multiplication factor.

Sperm Motility:

One hundred sperms on plain slide were examined and the numbers of progressively motile and immotile sperms were recorded. The motility percentage of sperms was calculated from the following formula:

Sperm motility was assessed by categorization into four groups of motility:

■Linear and rapid progressive (A) ≥25 µm /sec.

■ linear non rapid or rapid nonlinear (B) 5-24 µm/ sec.

■ Non progressive (C) <5 µm / sec.

■ Immotile (D) [11].

The sperm motility was counted by taking the percentage of forward progressive motile spermatozoa (grades A+B) which should be ≥50% of the total.

Sperm Morphology:

Spermatozoa were examined for normal and abnormal morphology using the same prepared slides for sperm motility. At least 100 spermatozoa were calculated, and normal sperm morphology (%) was assessed.

Oocytes collection:

Under sterile condition which includes surgical instruments sterilized by using autoclave and sterile operation site with the laminar air flow hood, the oocytes collection procedure done which start as following:Female mice were sacrificed by cervical dislocation at approximately 16-18 hrs post-hCG injection and the oviducts were isolated **.** For oocytes flushing, the ampulla was flushed the oviduct to release the oocytes cumulus masses using 0.5 mL SMART medium then, all the cumulus masses were transferred to a single fertilization dish by using a mouth-controlled pipette under aseptic conditions.Pipettes were used to pick up the oocytes, then they were transferred to a central well-culture dish containing 1 ml of culture medium (PH=7.4-7.6) and kept at 37°C with 5% CO2 and 95% humidity [12].

IVF Procedure:

The mature oocytes were washed twice in SMART medium and transported to 4-well culture dish (5-6 oocytes/ well) containing 1mL of the SMART medium either a lone (control group) or supplemented with one concentration of ATP (G2;1.25mM or G3;2.5mM). The motile spermatozoa were added to the oocytes at the concentration of approximately 5×10^4 motile sperm/oocyte.

Sperms and oocytes were covered with liquid paraffin and incubated at 36.5°C in the moist atmosphere 5% CO_2 with high humidity (95%) for 24h at CO_2 incubator. The percentages of IVF were recorded for every group [10].

Fertilization rates were assessed by recording the number of zygote, after

$$\text{Fertilization rate} = \frac{\text{No. of fertilized oocytes}}{\text{Total No. of oocytes}} \times 100$$ [12]

insemination and calculated according to the following formula:

Statistical analysis:

The data were statistically analyzed using SPSS/PC version 18 software (SPSS, Chicago). Fertilization Rate, embryonic development (%) and abnormal embryonic development (%) were analyzed using complete randomized design (CRD) (one way ANOVA).

Differences among means were computed using the Duncan multiple ranges test [13].

Results:

Oocytes collection:

In general, percentage of morphologically normal oocytes is (82.6 %) and morphologically abnormal oocytes are (18.4%). There was significant different (P<0.05) between both types of oocytes as shown in figure (1).

1-cell stage (zygote):

Figure (2) shows percentages of 1-cell embryo stage for the three groups. There was significant increment (P<0.05) between G1 and G2 groups. Also, significant increment (P<0.05) was obsereved between G3 and G2gruops. While, significant reduction (P<0.05) was shown between G1 and G3 groups.

A comparison was noticed among three groups for the percentages of abnormal embryonic development for 1-cells stage as shown in the figure (3). There was significant reduction (P<0.05) between G2 and G1groups. Mean while, non significant differences (P>0.05) were assessed between G3 and other two groups (G1and G2).

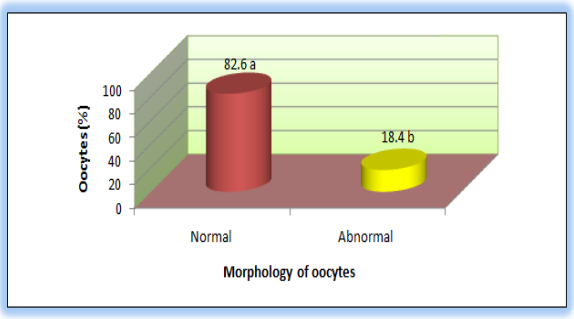

Figure (1): Percentages of normal and abnormal oocytes collected from female mice.

* Means with different superscripts within each columns are significantly different (P<0.05).

No. of normal oocytes: 730

No. of abnormal oocytes: 152

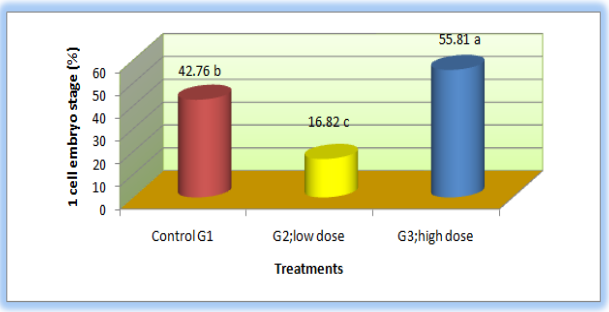

Figure (2): Percentages of 1-cell stage using SMART medium supplied with different concentrations of ATP.

* Means with different superscripts within each column are significantly different (P<0.05).

** Means with similar superscripts within each column are not significantly different (P>0.05).

Discussion

In the present study, using ATP with two concentrations (low concentration 1.25 mm and high concentration 2.5nm) were supplemented to SMART medium. An improvement was showed in the percentage of *in vitro* fertilization as compared to the control group. The present results were agreed with those found in studies done by Cummins (2002) [14], Chan (2005) [15] and Santos (2006) [16]they observed that the developmental potential of the embryo and the outcome of IVF have been shown to be related to both ATP content and mitochondrial content of human oocytes. Definitely, ATP a pharmacological agent that has gained much attention for increasing IVF success rates [17]. Another site, ATP content needed for the normal growth of embryo *in*

vitro, was not generated through embryonic cells but rather derived from the oocyte [18]. Actually, in the oocyte, ATP production depends mostly on the number and activity of mitochondria present in the ooplasm, which seem to be strongly related to developmental competence, with lower mitochondrial activity and number associated with premature arrest of the oocyte, fertilization failure, and reduced embryo development [19, 20].In present work revealed a significant different ($P<0.05$) in 1-cell embryo stage when using high concentration of ATP compared with other groups. These results may be return to increase in oxygen consumption observed at the time of first cleavage is associated with a higher energy demand for the initiation of the process of cell division [21,22].This increase in metabolic activity triggered by the first cleavage is likely to be a consequence of increased mitochondrial activity. Also, since mitochondrial oxidative phosphorylation is the major source of ATP production at this stage. On the other hand, as sperm motility is driven by the flagellum and is dependent on the availability of an adequate and continued supply of ATP [23]. And ATP was the main energy source used by the sperm flagellum to initiate and propagate forward motility [24]. Therefore, the sperm need high concentration of ATP to improve motility and stimulated fertilizing ability. This finding also is compatible with the finding of Foresta *et al.* [25, 26] revealed that in human spermatozoa, a concentration as high as 5 mM ATP possesses an important stimulatory action on fertilizing ability without any toxic or detrimental action on sperm motility and viability. this result goes with the finding of Zilli *et al.* [27]concluded that the relationship between ATP concentration and fertilization rate is due to the fact that the flagellar beat frequency of spermatozoa depends on ATP concentration and dynein ATPase activity which hydrolyses ATP to initiate motility.

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